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Extraction methods in soil phosphorus characterisation

- Limitations and applications

Helena Soinne

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Supervisor:

Prof. Helinä Hartikainen
Department of Applied Chemistry and Microbiology, University of Helsinki, Finland

Pre-examiners:

Prof. Elisabetta Barberis
Universita di Torino, Italia

Dr. Charles Shand
The Macaulay Institute, United Kingdom

Opponent

Dr. Gitte Holton Rubæk,
Department of Agroecology and Environment, University of Aarhus, Denmark

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Abstract

The quantification and characterisation of soil phosphorus (P) is of agricultural and environmental importance and different extraction methods are widely used to asses the bioavailability of P and to characterize soil P reserves. However, the large variety of extractants, pre-treatments and sample preparation procedures complicate the comparison of published results. In order to improve our understanding of the behaviour and cycling of P in soil, it is crucial to know the scientific relevance of the methods used for various purposes. The knowledge of the factors affecting the analytical outcome is a prerequisite for justified interpretation of the results. The aim of this thesis was to study the effects of sample preparation procedures on soil P and to determine the dependence of the recovered P pool on the chemical nature of extractants.

Sampling is a critical step in soil testing and sampling strategy is dependent on the land-use history and the purpose of sampling. This study revealed that pre-treatments changed soil properties and air-drying was found to affect soil P, particularly extractable organic P, by disrupting organic matter. This was evidenced by an increase in the water-extractable small-sized (<0.2 µm) P that, at least partly, took place at the expense of the large-sized (>0.2 µm) P. However, freezing induced only insignificant changes and thus, freezing can be taken to be a suitable method for storing soils from the boreal zone that naturally undergo periodic freezing.

The results demonstrated that chemical nature of the extractant affects its sensitivity to detect changes in soil P solubility. Buffered extractants obscured the alterations in P solubility induced by pH changes; however, water extraction, though sensitive to physicochemical changes, can be used to reveal short term changes in soil P solubility. As for the organic P, the analysis was found to be sensitive to the sample preparation procedures: filtering may leave a large proportion of extractable organic P undetected, whereas the outcome of centrifugation was found to be affected by the ionic strength of the extractant. Widely used sequential fractionation procedures proved to be able to detect land-use -derived differences in the distribution of P among fractions of different solubilities. However, interpretation of the results from extraction experiments requires better understanding of the biogeochemical function of the recovered P fraction in the P cycle in differently managed soils under dissimilar climatic conditions.

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Helsinki, October 2009

Helena Soinne

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List of original publications

This thesis is based on the following publications which are referred to in the text by their Roman numerals:

I Peltovuori, T. & Soinne, H. 2005. Phosphorus solubility and sorption in frozen, air-dried, and field-moist soil. *European Journal of Soil Science* **56,** 821–826.

II Soinne, H., Räty, M. & Hartikainen, H. Effect of air-drying on phosphorus fractions in clay soil. Accepted to *Journal of Plant Nutrition and Soil Science*.

III Soinne, H. & Peltovuori, T. 2005. Extractability of slurry and fertilizer phosphorus in soil after repeated freezing. *Agricultural and Food Science* **14,** 181–188.

IV Soinne, H., Saarijarvi, K., Karppinen, M. & Hartikainen, H. 2008. Sensitivity of soil phosphorus tests in predicting the potential risk of phosphorus loss from pasture soil. *Agricultural and Food Science* **17,** 265–277.

V Soinne, H., Uusitalo, R., Sarvi, M., Turtola, E. & Hartikainen, H. Characterisation of soil phosphorus with chemical extraction methods and ^{31}P-NMR spectroscopy in differently managed clay soil. Manuscript.

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Author's contribution to the articles

I The experimental design was originally planned by Tommi Peltovuori. Helena Soinne refined the plan and conducted the laboratory work. Both authors took part in interpreting the results. The manuscript was prepared by Tommi Peltovuori and Helena Soinne commented on it.

II Helena Soinne planned the study along with the other co-authors. The laboratory work was conducted jointly by Helena Soinne and Mari Räty. Helena Soinne prepared the manuscript and Mari Räty commented on it. Helinä Hartikainen contributed with conclusions and comments.

III The experimental design was planned jointly by Tommi Peltovuori and Helena Soinne. Most of the laboratory work was conducted by Helena Soinne. Both authors took part in interpreting the results and the manuscript was prepared jointly by Tommi Peltovuori and Helena Soinne.

IV The original idea for the paper was developed by Helena Soinne together with the co-authors in the project "The role of organic phosphorus in plant nutrition and water loading" conducted by Helinä Hartikainen. Kirsi Saarijärvi was responsible for the field work and Helena Soinne performed the laboratory analyses. Helena Soinne prepared the manuscript and Kirsi Saarijärvi wrote the description of the experimental design. Minna Karppinen (now Sarvi) and Helinä Hartikainen commented on the manuscript.

V The original idea for the paper was developed by Helena Soinne together with the co-authors in the project "The role of organic phosphorus in plant nutrition and water loading" conducted by Helinä Hartikainen. Helena Soinne performed the laboratory analyses with Minna Sarvi (née Karppinen). Risto Uusitalo was responsible for interpreting the ^{31}P-NMR spectra. Helena Soinne prepared the manuscript, and Helinä Hartikainen, Minna Sarvi, Risto Uusitalo, and Eila Turtola commented on it.

1 Introduction

In later half of 19th century, papers dealing with phosphorus (P) retention in soil and extraction of P from soils were already published (Kurtz, 1953). Being an essential nutrient for plant growth, the estimation of plant-available P has a long history. According to Kurtz (1953), in the beginning of the 20th century the scientific community was interested in developing a chemical extraction method for predicting the crop responses to P fertilisation. Since then, knowledge concerning soil P has increased greatly and the methods used for its characterisation have developed further to produce more detailed information on soil P forms.

The natural state of soil P, regulated by pedogenesis, can be affected by soil use and management. The P cycle defined by slow weathering processes and biological transformations is disturbed in agricultural fields by the removal of P with the crop and, on the other hand, by adding large amounts of P at once into the cycle. Concomitant with the long-term weathering processes and human-induced changes in the P pools, there are relatively fast changes related to seasonal variation and crop growth. These faster changes are brought on by environmental factors, such as moisture and temperature that affect biological activity. Seasonal variation in biological activity is reflected in labile P forms in soil.

Environmentally and agronomically, the P pool that is of the most interest in soil is the easily soluble P that is considered to be equal to the plant available P (labile P). To measure the labile P, many routine extraction tests have been developed and the results of these tests guide soil fertilisation. However, as the samples are taken into the laboratory and pre-treated for routine analysis, the natural processes in soil material are disturbed and the measured P is no longer directly related to the situation and ongoing processes in the field. The changes in soil P taking place between the soil sampling and the analysis of P concentration can be qualitative or quantitative and their importance will depend on the purpose of the analysis. In this study, the effect of procedures used for routine P analysis, the impact of the sampling system and pre-treatment and the chemical characteristics of extractants on the P value measured in the laboratory are discussed.

1.1 Forms and reactions of phosphorus in soil

Phosphorus occurs in soil in several inorganic and organic forms. Inorganic phosphorus (P_i) includes apatitic minerals, secondary precipitates formed with Ca, Fe and Al and free phosphate ions ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-} all denoted here as PO_4-P) attached to sorption surfaces or dissolved in the soil water. Organic phosphorus (P_o) includes a group of organic molecules having P as a part of their structure. Orthophosphate esters are P_o compounds having an ester linkage joining PO_4-P to organic moiety and they are further divided into mono- and diesters according to the number of ester groups attached to each PO_4-P. The largest group of P_o in most soils is the monoesters (Turner et al., 2002), including sugar phosphates, phosphoproteins, mononucleotides and inositol phosphates.

Diesters including nucleic acids, phospholipids, teichoic acid and aromatic compounds generally occur in smaller concentrations in soil than monoesters. Phosphonates form a group of P_o that contain a C-P bond. Polyphosphates complicate the simple division of P into organic and inorganic pools, since most of them are inorganic but are of biological origin. However, polyphosphates such as ADP and ATP are, chemically, organic compounds.

The reactions controlling the P cycle in soil include solubilisation, precipitation, sorption, desorption, leaching, immobilisation and mineralisation. Apatitic P, the primary sources of P in soils, is solubilised through weathering processes. The ionic form of solubilised P depends on the pH of the solution, the predominating species in slightly acidic soils being $H_2PO_4^-$ and that in soils having a pH over 7 being HPO_4^{2-}. Solubilised P can be leached from the soil, sorbed onto Fe and Al oxides and mineral edges of clay particles, precipitate as secondary Ca-, Fe- or Al-minerals or be taken up by living organisms. Only a small proportion of soluble P is dissolved in soil water. The negatively charged PO_4-P can be bound to positively charged surfaces through electrostatic forces; however, the importance of this mechanism is minimal in soil environments compared to ligand exchange.

Ligand exchange, originally discovered in 1967 by Hingston et al. allows fairly strong sorption of P and is considered to be the main retention mechanism for P_i in non-calcareous soils. In this reaction, PO_4-P is chemisorbed onto variably-charged sorption components. Ligand exchange refers to the formation of an inner-sphere complex where PO_4 group displaces through its oxygen-bearing group an OH^- or H_2O group from Al or Fe in a hydrous oxide or on the edge surface of a clay mineral. The reaction can take place on a positively, negatively or uncharged surface. However, the H_2O ligand, predominant in acidic conditions, increases the positive charge on a sorption surface and is easier to replace with a PO_4 group (Rajan et al., 1974), thus enhancing the sorption of P at lower pH. The PO_4-P in soil solution tries to reach equilibrium with PO_4-P sorbed onto soil surfaces. Therefore, an increase in PO_4-P concentration in soil solution enhances P sorption. At lower concentrations, the formation of binuclear or bidentate complexes, where PO_4 group displaces two coordinated OH^- or H_2O groups from a hydrated surface, is favoured, but as the level of adsorption increases the formation of monodentate structures is preferred and the sorption strength is decreased (White, 1979). The fast adsorption of PO_4-P onto variably-charged soil oxides and hydroxides is followed by slower diffusion of P into the hydroxide (Barrow, 1983). This diffusion proceeds with time, decreasing the plant availability of P (Barrow, 1979a; 1983).

Desorption of P is enhanced in conditions opposite to those favouring sorption. In increasing pH, desorption is enhanced and similarly, a decrease in the PO_4-P in the soil solution enhances P desorption from the sorption sites. Further, sorption and desorption of P is affected by P concentration and ionic strength in the soil solution and temperature (Barrow, 1983). An increase in salt concentration increases the sorption of P (Ryden & Syers, 1975) and decreases the desorption (Hartikainen & Yli-Halla, 1982). The rate of sorption and desorption of P increases with temperature (Barrow, 1979b).

Precipitation can be seen as a reverse reaction to mineral dissolution and can be defined as formation of discrete, insoluble compounds in soil (Pierzynski et al., 2005). In

alkaline soils where Ca is the predominant cation, the soluble PO_4-P can precipitate, forming Ca phosphates. In these soils, the dicalcium phosphate dihydrate ($CaHPO_4 \cdot 2H_2O$) which forms readily after the addition of P to soil, can in the long term turn into more stable forms of calcium phosphates (Freeman & Rowell, 1981; Pierzynski et al., 2005). In acidic soils, Ca phosphates are unstable and in favourable conditions P can form precipitates with Al and Fe instead of Ca. Separate crystals of Al and Fe phosphates have been detected (Martin et al., 1988), and also evidence of the surface precipitation of P has been reported (Ler & Stanforth, 2003). However, some studies support the idea that the slow diffusion of P into the pores of amorphous oxides is the reaction mechanism rather than precipitation (Magid & De Arambarri, 1985), even though sorption is thought to proceed to precipitation in the long run (Pierzynski et al., 2005).

Inorganic P is transformed and incorporated into the organic pool through the biological cycle. Plants and microbes take up P required for their growth and after the death of living organisms, the immobilised P returns to the soil through mineralisation by microbes. The microbiological decomposition process results in a release of both P_i and P_o. Of P_o compounds, esters can be adsorbed onto soil sorption surfaces through the PO_4 group by a similar mechanism to that for P_i. Diesters have only one ionisable proton per PO_4 group compared to two ionisable protons in monoester PO_4 groups and, thus, are less strongly sorbed onto soil surfaces. Lacking the sorption-induced protection, the diesters are more easily mineralised than monoesters (Celi & Barberis, 2005). Guan et al. (2006) reported *myo*-inositol hexaphosphates, monoesters which contain six PO_4 groups to adsorb on Al-hydroxides with three PO_4 groups and Celi et al. (1999) on goethite with four of its six groups. The strong sorption protects inositol hexaphosphates from mineralisation and leads to their accumulation in soil (Steward & Tiessen, 1987; Celi et al., 1999). Further, inositol hexaphosphates have been reported to displace inorganic PO_4-P from sorption sites (Bowman et al., 1967; Anderson et al., 1974; Berg & Joern, 2006)

In addition to sorption to Fe and Al oxides and mineral edges of clay particles, P_i and P_o can be bound to organic molecules through cationic bridges (Gerke & Hermann, 1992). Attempts have been made to categorise the P associated with organic matter through cationic bridges by fractionating organic matter to fulvic and humic acids with sequential alkali and acid extractions. According to Makarov and Malysheva (2006) in acid soil, more P is associated with humic acids, whereas in calcareous soils, associations with fulvic acids are dominating. However, the fractionation of fulvic and humic acids is sensitive to methodological biases and redistribution of P during the extraction procedure may occur (Makarov & Malysheva, 2006).

In some studies, the mobility of P_o compounds has been found to be greater than that of the inorganic forms (Chardon et al., 1997) and the role of P_o in providing plant-available P through mineralisation is well established (Haas et al., 1961; Sharpley, 1985; Stewart & Tiessen, 1987). These findings of strong sorption and, on the other hand, of high mobility reflect the heterogeneity of the P_o pool and its behaviour. Soil P_o content is closely related to soil organic C (Kaila, 1963b; Guggenberger et al., 1996; Bünemann et al., 2006), and therefore it is obvious that organic matter turn-over and microbial activity largely dictate the reactions of P_o in soil.

1.2 Phosphorus cycling in agricultural fields

In soil, P is constantly cycling, mediated by the biological P requirements of all living organisms. When soil is harnessed for agricultural production the natural cycling will be disturbed by fertiliser additions and removal of nutrients with harvesting. The effects of human activity on soil P have been studied for quite some time. Compared to virgin surface soils, the total P (TP) in cropped soils not receiving any fertiliser decreases (Schollenberger, 1920; Haas et al., 1961), whereas in fields receiving P fertilisation, the TP in the surface soil tends to increase (Haas et al., 1961; Kaila, 1963c).

For long term sustainability of cropping, adequate amounts of P through fertilisation are needed. The addition of inorganic P fertilisers has been reported to increase the concentration of P_i in soil (Kaila, 1964b; Perrot et al., 1990). Phosphorus fertilisation given in manure, on the other hand, has been found to increase the concentration of P_o (Sharpley et al., 2004) but results showing an increase in P_i concentrations have also been published (Haas et al., 1961; Uusitalo et al., 2007). Guggenberger et al. (2000) found that fertilisation increased the labile P_o pool, but the type of fertiliser seemed not to have any importance and Otabbong et al. (1997) concluded that in soils well supplied with available P, fertiliser additions especially increase the P_o concentration. These findings on the response of soil P pools to fertiliser additions are not unambiguous and support the findings of Salonen (1946), who suggested that the relative amount of P_o in soil is related to soil texture rather than fertilisation and Rubæk and Sibbesen (1995) who concluded that the level of soil P_o depends more upon the cropping system and tillage intensity than on the fertiliser used.

McLauchlan (2006) reviewed the impacts of agricultural practices on soil properties and concluded that tillage and harvesting tend to decrease concentrations of organic matter and nutrients in soil. Due to the fairly close relationship between organic C and P_o (Kaila, 1963a; Guggenberger et al., 1996; Bünemann et al., 2006), management practices increasing soil organic matter are expected to increase soil P_o concentrations. Compared to conventionally tilled soil, in no-tilled fields, organic C and available P has been found to be enriched in surface soils (e.g. Robbins & Voss, 1991; Selles et al., 1999; Muukkonen et al., 2007). The higher concentrations of available P in the surface soil in no-tilled soil have been suggested to originate from non-incorporated surface application of P fertilisers and from the high amount of crop residues left to decompose at the soil surface (Robbins & Voss, 1991; Janzen et al., 1992). According to Muukkonen et al. (2007), the accumulation of organic C may enhance availability of P in clay soils due to the competition between organic anions and PO_4-P for the same sorption sites.

In addition to fertilisation- and tillage-induced changes in P_i and P_o, the P cycle in agricultural fields is subjected to seasonal changes. In fertilised soils, the seasonal variation in P has been suggested to result from physicochemical changes in soil induced by management practices, such as fertilisation, but in non-fertilised soils it is attributable to biological cycling of P (Sharpley et al., 1995; Styles & Coxon, 2007). The major alterations are caused by accumulation of labile P forms during the winter, and their decrease during the growing season (Saunders & Metson, 1971; Sorn-Srivichai et al., 1988; Fabre et al., 1996). In temperate regions, P_o especially builds up during the winter

(Tate et al., 1991). Sorn-Srivichai et al. (1988) suggested that the utilisation of soil P during the winter months is relatively low because of the low soil temperature, which retard plant growth. As the weather gets warmer in the spring, the accumulated labile P_o is mineralised along with the increased microbial activity and the solubilised P will meet the needs of the fast growing plants (Chen et al., 2003). Turner and Haygarth (2000) reported springtime maximum in P leaching and suggested the increase to originate from microbial biomass. In the boreal zone, the growing season is shorter than at lower latitudes, and during the winter months the soil is at least part of the time frozen and the biological activity in soils is minimal. In incubation studies, freezing has been found to increase the solubility of soil P, especially in high organic matter soils (Ron Vaz et al., 1994); however, freeze-thaw studies have been criticised because of unnatural freezing rates and minimum temperatures (Henry, 2007). According to Yli-Halla et al. (1995) the higher concentrations of P in runoff water in autumn and early spring most likely originate from decaying plants.

1.3 Characterisation of soil phosphorus

Extraction methods aim to categorise soil P into different pools that respond similarly to an extractant-induced change in chemical environment. Single extractions are used to give an estimate of a certain pool of P whereas sequential extractions aim to characterise P in more detail and separate P into pools that are separated by some chemical property. Extraction methods used in soil P characterisation are based on 1) desorbing soil P from the sorption sites by creating circumstances where desorption is enhanced, 2) replacement of soil P with a compound having a stronger sorption affinity or 3) solubilisation of sorption components.

1.3.1 Identification of chemical forms

Almost every publication on soil P reserves reports the total P (TP) concentration. However, there are only a few methods that actually give the absolute total concentration in soil. To measure the absolute total amount of P in soil, the organic matter in a sample needs to be oxidised and the mineral matrix needs to be disintegrated and dissolved. However, to dissolve all the mineral constituents in the sample, very aggressive treatments are required and thus, the total amount of P in soil can not be recovered using only extraction methods. Even though TP can not be recovered by extraction, high amounts of soil P can be solubilised from the soil using strong acids or bases.

The commonly used methods for TP determination exploit heating with one or a mixture of strong acids. However, the alkaline Na_2CO_3 fusion has been found to give the highest recovery of P, and according to Syers et al. (1967) it is the most effective method for samples containing apatite inclusions. Bowman (1988) reported that $HClO_4$ digestion can recover on average 92% of the TP measured with Na_2CO_3 fusion, but according to Syers et al. (1967) it underestimates TP, especially in strongly weathered soils.

The methods that aim to categorise soil P according to its chemical forms are based on the known selective properties of certain chemicals for extracting a fairly uniform pool that represents a proportion of soil total P. At its simplest, the characterisation based on chemical form aims to separate organic and inorganic forms of P. However, in practice, the separation of inorganic and organic forms of P appears to involve many uncertainties, of which the indirect way to calculate the P_o as a difference between TP and P_i is not the least.

Soil P_o can be estimated by burning the organic matter, whereupon the P_o equals the increase in P_i concentration, or by using extractants that are known to efficiently extract soil organic matter. Because there has been no direct way to analyse P_o concentrations, the P_o is calculated as a difference between TP and P_i in the extract and is thus prone to errors. The ignition method developed by Saunders and Williams (1955) and modified by Walker and Adams (1958) are found to overestimate the concentration of total P_o (TP_o) in soil. The overestimation may originate from the increased extractability of P_i at temperatures above 160^{o}C (Williams et al., 1970) or from the possibility that during the acid extraction of the unignited sample, P_i associated with organic matter through metal bridges will end up as a part of TP_o (Oniani et al., 1973). However, Williams et al. (1970) concluded that the ignition methods are fairly reliable for weakly weathered soils. Sodium hydroxide (NaOH) is one of the oldest and most used methods for extracting soil organic matter. At high pH, many organic functional groups are ionized and the increased charge density leads to increased solubility. In NaOH extraction the stability of organic molecules also decreases as Na^+ replaces polyvalent cations, causing swelling and dispersal of clays and organic material (Oades, 1988; Swift, 1996).

Classical extraction methods for P_o, like the one developed by Mehta et al. (1954), employ acid as the primary extractant along with subsequent alkali extraction. The mineral acid treatment increases the solubility of organic matter by removing bridging cations and dissolving salts of organic phosphate esters (Turner et al., 2005). From the combined extracts, concentrations of P_i and TP are determined and P_o is calculated as the difference between TP and P_i.

The sequential acid and base extractions being tedious, Bowman and Moir (1993) proposed a single EDTA-NaOH extraction for P_o determination. The addition of EDTA, which chelates metal cations, increases the solubility of P bound via cationic bridges and Bowman and Moir (1993) reported the extractability of P_i in NaOH-EDTA to generally increase as the proportion of Na_2EDTA in relation to NaOH in the extractant increases. The recovery of P with NaOH-EDTA has been reported to vary from 45% to 88% of the TP for permanent temperate pasture soils (Turner et al., 2003d). In some studies the NaOH-EDTA has been reported to extract over 90% of TP from high organic matter soils or from organic forest soil layers (Cade-Menun et al., 2000; Turner et al., 2003a; Turner et al., 2004) but, on the other hand, lower recoveries are found for soils high in acid-extractable P_i (Turner et al., 2006).

During the extractions, the organic matter may undergo structural changes. The hydrolysis of labile P_o during the NaOH extraction has been suggested to lead to overestimation of P_i (Guggenberger et al., 1996). However, inositol phosphates and diesters have been reported to hydrolyse to lower phosphate esters and therefore the total

amount of P_i or P_o would not be affected (Bowman, 1989; Turner et al., 2005). Acid hydrolysis, on the other hand, releases PO_4-P leading to underestimation of the P_o concentration (Hens & Merckx, 2002; Turner et al., 2005).

For decades the P_o has only been estimated as the difference between total P and P_i in soil, but in recent years, ^{31}P-NMR spectrometry has enabled more detailed descriptions of P_o forms in soil. Nonetheless, the solution NMR spectroscopy, most often used in soil analysis still requires extraction of soil P into solution. Cade-Menun and Preston (1996) found the single extraction method developed by Bowman and Moir (1993) to be suitable for NMR analysis, but they concluded that any extractant used in ^{31}P-NMR studies will influence the amount and composition of P detected.

To characterise soil P_i in detail Chang and Jackson (1957) developed a sequential fractionation procedure based on the ability of selected extractants to distinguish between P in different pools. Originally their procedure was thought to separately solubilise P in discrete minerals. The discovery of ligand exchange (Hingston et al., 1967) created a new theoretical basis for this approach. In the light of current knowledge, it is clear that the procedure that Chang and Jackson (1957) developed separates the different P_i pools according to the surface the P is bound to. The first extraction step aims to remove the loosely bound P and exchangeable Ca^{2+}, possibly interfering with the subsequent extraction steps. The use of NH_4F in the second extraction step is based on the ability of fluoride to form a stable complex with Al and to selectively extract Al-bound P (Turner & Rice, 1952; Chang & Jackson, 1957). On the other hand, contradictory results on the ability of NH_4F to specifically extract Al-bound P have been reported (Bromfield, 1967). The subsequent NaOH extraction is done to remove Fe-bound P and the final extraction step, done with H_2SO_4, is assumed to attack Ca-bound P. Reductant-soluble P is solubilised during heating with 0.3 M Na-citrate and 1 g of $Na_2S_2O_4$, and residual P is calculated as the difference between TP and the sum of all fractions. Several modifications of the fractionation procedure have been published (e.g. Fife, 1959; Williams et al., 1967; Hartikainen, 1979). Williams et al. (1967) discovered that the Chang and Jackson fractionation procedure was not applicable to calcareous soils or sediments and based on their work a fractionation procedure for calcareous soils was developed further. The Chang and Jackson procedure and its modifications have been used for characterisation of Finnish soils e.g. by Kaila (1964a, b), Hartikainen (1979) and Peltovuori (2006). Kaila (1964a) found the proportions of reductant-soluble and residual P to be insignificant in poorly developed Finnish soils, and thus, these fractions have not been determined in most of the subsequent studies.

Another widely used fractionation procedure was developed by Hedley et al. (1982). Their method aims to categorise P into pools based on biological availability. In Hedley procedure, P_o is also taken into account. The P_i and P_o extracted with stronger solutions are assumed to represent less bio-available P pools than the preceding fractions extracted with milder solutions. As with the Chang and Jackson procedure, the first step in Hedley fractionation extracts loosely bound P from soil. The second extraction is done with $NaHCO_3$ which has been suggested to give an estimate of plant available P for calcareous soils (Olsen et al., 1954). Even though the bicarbonate extraction was developed for calcareous soils having a relatively high pH, it has been used for many type of soils as a

part of Hedley fractionation procedure. These first two fractions constitute the labile and readily plant available fraction of P, and the next two extractions, done with NaOH and HCl, represent P pools bound more strongly to soil. The residue is digested with H_2SO_4 and H_2O_2 to recover the residual P (Hedley et al., 1982). The procedure was further modified by Condron et al. (1990), who added another NaOH extraction to the end of acid extraction to solubilise the occluded P. Cross and Schlesinger (1995) classified the Hedley fractions into primarily biological and geochemical fraction, where the biological P is represented by $NaHCO_3$- and NaOH-extractable P_o, and the sum of all P_i extracted represents the geochemical pool.

1.3.2 Extracting plant-available phosphorus

In the beginning of the 20th century it was generally recognised that the total soil P content does not define the plant available P in soil (Kurtz, 1953). Nelson et al. (1953) reviewed routine soil test procedures used in estimating P plant availability and divided them into two broad categories: 1) extraction methods and 2) biological methods. Because the biological methods, involving field experiments or greenhouse experiments with higher plants or use of microbes as indicators for available P were too time consuming to be used as routine tests, the development of extraction procedures expanded. In 1953, the chemical extraction methods used formed a long list, including water, carbon dioxide-saturated water, acids, bases, salts, buffered solutions, electrodialysis and ion exchangers (Nelson et al., 1953). A large variety of extraction tests is used all over the world and actually, in Scandinavian countries, five different procedures for extracting plant-available P from soil are used (Krogstad et al., 2008). Behind each procedure in use, there are extensive studies on the ability of the method to produce P concentrations that correlate well with the P uptake by plants and are thus calibrated with fertilisation recommendations. However, the extraction tests used for bio-availability estimations of P often serve at the same time as tests to estimate the availability of other important nutrients and might even be developed to extract other nutrients from soil. In these cases the theory and mechanism behind the solubilisation of P is not always clear.

Water is probably the simplest extractant used for plant available P and it is used as a soil P-test in Netherlands and in Germany (Vanderdeelen, 2002). Olsen et al. (1954) suggested bicarbonate extraction for predicting plant availability of P in calcareous soils. In calcareous soils, the main function of $NaHCO_3$ in extracting P is to decrease the Ca^{2+} activity by forming $CaCO_3$ (Olsen et al., 1954). However, in acid soils it is more likely that the solution pH, buffered to 8.5, promotes desorption of P. In soils, containing Al- and Fe-bound P, the P concentration in solution increases as the pH increases because at high pH, the higher concentration of OH^- ions decreases the ability of PO_4-P to compete for sorption sites (Rajan et al., 1974; Hartikainen, 1981; Hartikainen & Yli-Halla, 1996). Olsen's bicarbonate method is still widely used in estimating the soil plant availability e.g. in Denmark, England, Australia and New Zealand (Vanderdeelen, 2002).

Other extractants suggested for soil P testing include organic acids that were assumed to simulate organic acids excreted by plant roots, and mineral acids that, according to

Nelson et al. (1953), were originally selected largely based on the analytical expediency. The organic acids used are citric acid, lactic acid and acetic acid, and methods exploiting these are used in the EU at least in Austria, Belgium, Finland, France, Germany, Ireland (Vanderdeelen, 2002) and Sweden. In the United States of America, the widely used Mehlich-1 test uses 0.05 M HCl and 0.0125 M H_2SO_4. In addition to different chemical extractants, methods simulating P uptake by plant roots have been developed. Anion-exchange resins and iron-impregnated strips can act as sinks for P, and thus, mimic plant uptake. Thus, methods and extractants intended for the same purpose, such as estimating soil plant available P, can be expected to and do give different results for the same soil (see Neyround & Lischer, 2003). However, the fertilisation recommendations are adjusted separately for each country and each test.

While the soil test P (STP) measured in most routine procedures is inorganic, the role of soil P_o in plant nutrition is widely recognised. In 1951, Eid et al. already suggested that the accuracy of chemical soil tests for P availability would be improved by taking into account the appropriate P_o fraction. However, because the tests were originally designed for P_i measurements, the analysis of P_o from these extractions is not always straightforward and may not be of any relevance. Bowman and Cole (1978) found $NaHCO_3$ extraction, which is used for estimating the amount of plant-available P_i, to be suitable for evaluating the plant-available P_o in soil, whereas Hayes et al. (2000) concluded that only a small proportion (1-9%) of bicarbonate-extractable P_o is labile.

According to Nelson et al. (1953), the development of soil test methods is complicated by the fact that plants differ in their ability to obtain P from soil. Although the chemical form of P in soil largely defines the potential biological availability, there are several processes that affect the quantity that can be taken up by plants. In 1965, Sauchelli reviewed limitations behind chemical soil tests and concluded that the results of soil tests reflect the prevailing soil conditions only at one brief moment of the soil's dynamic and biotic sequence of events. Therefore, the important part of soil testing is to know how to interpret the results in a practical way (Sauchelli, 1965).

1.3.3 Estimating the risk for soil phosphorus loss

In recent decades the need for soil P research has arisen from environmental factors. Due to the eutrophication of watercourses, the EU and governments have started to regulate P fertilisation. Already in 1955 there were fields in the USA that were enriched with P (Millar, 1955). Sauchelli described in 1965 the loss of fertiliser-P with eroded material, but it was not until the late 1960's that the eutrophication of lakes turned a new page in soil P research.

In literature published before the 1970's the soil tests are reviewed only from the perspective of plant availability but since then, positive correlation between STP and P concentration in the drainage water has been established for many of the above mentioned methods (see Pote et al., 1996; Turtola & yli-Halla, 1999). However, a high STP value does not automatically cause eutrophication of nearby waters. Thus, when assessing the risk for P loss, in addition to the P concentration in the field, the transport of P should also

be considered. Phosphorus index is an example of a tool developed to asses the risk of P loss, taking into account the P source, P applications as manure and fertiliser, and transport factors.

1.3.4 Analysis of extracted inorganic and organic phosphorus

Measurement of the P amount transferred to the solution phase during the extraction can be done colorimetrically or by using inductively coupled plasma (ICP) spectroscopy. The ICP determination gives the total amount of P in solution and thus, to analyse P_i and P_o separately requires the use of colorimetric methods. The most commonly used molybdenum blue method, developed by Murphy and Riley (1962), is based on the reaction of PO_4-P with molybdate to form a blue compound at low pH in reducing conditions. The intensity of the colour corresponds to the concentration in the solution and can be measured with a spectrophotometer. The concentration of TP in the solution can be determined colorimetrically after digestion of the sample, e.g. in autoclave with oxidising chemical, such as persulfate ($K_2S_2O_8$). The quantity of P_o in the extracts is derived as a difference between TP and P_i.

In molybdenum blue method, the coloured compound is formed with free PO_4-P and the resulting concentration is thought to represent the dissolved P_i. However, problems involved in measuring dissolved P_i with the molybdenum blue method were acknowledged already in 1968 when Rigler hypothesised that the molybdenum blue methods give values for dissolved PO_4-P that are too high because of the hydrolysis of P_o taking place in the acidic conditions required for molybdenum blue complex formation. Turner et al. (2005), however, concluded that the error caused by acid hydrolysis is likely to be negligible in quantitative analysis and, in fact, reported molybdenum blue method to give too low values for PO_4-P in solution because of PO_4-P associated with organic molecules may precipitate in acidic conditions during the colour formation. Yet another source of error in the analytical procedure for measuring the P_i with the molybdenum blue method results from the fact that the intensity of the colour reflects only the amount of phosphate phosphorus (PO_4-P) in the sample leaving the other P_i compounds, such as poly- and pyrophosphates, undetected (Turner et al., 2005). This kind of underestimation of P_i concentration by molybdenum blue method can be demonstrated by using ^{31}P-NMR spectroscopy (Turner et al., 2003d; Turner et al., 2006).

Traditionally, membrane filtration with a pore size of 0.45 μm or 0.2 μm has been used to divide the P in solution to dissolved and particulate fractions. However, the so-called dissolved fraction contains significant amounts of colloidal-size P (Hens & Merckx, 2002; Koopmans et al., 2005). In acidic conditions, the PO_4-P bound to colloidal-size organo-mineral complexes may be released into the solution through dissociation of complexes (Gerke & Jungk, 1991) leading to an overestimation of dissolved PO_4-P (Negrin et al., 1995; Turner et al., 2003d). On the other hand, the P_o concentration in solution may be overestimated at the expense of P_i because the PO_4-P bound to organic matter or colloidal-size particles does not react to form a coloured complex. To minimise colloidal disturbance, Hens and Merckx (2002) suggested using filters having a pore-size of 0.025

µm because, according to their findings, free PO_4-P was the only P species passing through this type of membrane. The filtration, however, does not support the analysis of P_o solubilised during the extraction and Gimbert et al. (2005), who detected that filtration removed a significant proportion of the colloidal fraction, suggested that centrifugation should be preferred when studying colloidal material in soil suspensions.

Because of the uncertainties in analysing P_i with the molybdenum blue method, it has been advised that the resulting concentration be called molybdate-reactive P (MRP) and the difference between total P and MRP be called molybdate-unreactive P (MUP) instead of organic P (see Haygarth & Sharpley, 2000).

1.4 Aims of this study

The quantification and characterisation of soil P is of agricultural and environmental importance. The use of extraction methods does not require refined analytical equipment and is relatively inexpensive; thus, these methods are not likely to be replaced, despite the constant development of more sophisticated methods. Different P forms differ in their bioavailability and their potential to cause detrimental P loading when ending up into watercourses. In order to improve our understanding of P behaviour, we need to understand better the scientific basis of the extraction methods used for various purposes; in particular, we need to identify the factors that affect analytical results, and consequently, their interpretation. The factors studied in this thesis were based on the following reasoning:

- The analyses of samples taken into the laboratory are expected to produce results corresponding to the prevailing conditions in the soil. However, in most cases, it is not possible to analyse the samples right away; thus, samples are stored prior to analysis. One objective of this thesis was to study the pre-treatment -induced changes in soil P characteristics (I, II, III).

- The biological activity in soil varies with the season and soil temperature; both can affect the biogeochemical cycling of P. In this thesis, the importance of sampling time on the interpretation of the results is evaluated (III). Furthermore, the sensitivity of soil P tests to changes in environmental conditions are discussed comparing environmental and agricultural perspectives (IV).

- There is a large variety of extractants used in soil P studies. Different pH or ionic strengths of extractants can be expected to affect the amounts of P_i and P_o extracted. In this thesis, extraction procedures and the chemical nature of the extractants were studied to determine their effects on solubilised P_i and P_o (II, IV, and V). One objective was to compare different extraction methods that categorise soil P differently for their ability to detect management-induced changes in soil P pools (V).

2 Material and methods

2.1 Soil samples

The properties of soil samples used in the original publications are compiled in Table 1. The effect of sample storage was studied with two fine-textured (Sjökulla and Kotkanoja) and two coarse (Loppi and Toholampi) cultivated mineral soils (I). The samples were taken from the Ap horizon and passed through a 5-mm sieve. Then they were divided into two subsamples to be stored field moist at +5°C or air-dried at room temperature (ca +20°C).

Table 1. Soil samples, sampling depths ans soil characteristics.

Soil	Used in	Sampling depth (cm)	pH 1:2.5	Clay %	C %	STP* mg l^{-1} soil	Soil P class**
Loppi	**I**	**0-30**	6.7a	9	3.6	88.3	excessive
Toholampi	**I**	**0-27**	5.0a	4	3.0	6.5	fair
Kotkanoja	**I**	**0-24**	6.5a	48	2.5	11.2	satisfactory
Sjökulla	**I**	**0-20**	4.4a	49	2.1	6.2	fair
Unmanaged grassland	**II, V**	**0-20**	5.7b	60	5.2	<1	poor
Organically cultivated	**II, V**	**0-20**	6.0b	61	4.4	1.4	poor
Conventionally cultivated	**II, V**	**0-20**	5.7b	65	3.6	2.3	rather poor
Organic soil	**III**	**0-32**	6.4a	24	12.8	17.5 mg kg^{-1}	good
Mineral soil	**III**	**0-33**	7.3a	25	1.7	18.5 mg kg^{-1}	good
Pasture land* **	**IV**	**0-2**	5.5b	6.9	3.0	12.9	satisfactory
		2-10	5.7b	6.9	3.0	9.1	satisfactory

a) Determined in 0.01M $CaCl_2$

b) Determined in water suspension

* Acid ammonium acetate extraction (Vuorinen & Mäkitie, 1955)

** According to Finnish guidelines the soils can be divided into seven categories according to their P status: poor, rather poor, fair, satisfactory, good, high and excessive

*** At the beginning of the experiment

The effect of drying on the soil P fractions (II) was further studied with fine-textured soil collected from the Yöni experimental farm managed by MTT Agrifood Research Finland at Jokioinen. At the beginning of the 20th century, this whole study area of 1.5 ha had been an unmanaged grassland. In 1991, parts of the grassland were taken for organic and conventional cultivation. For the organically and conventionally cultivated soils the tillage and crop rotations were similar: ploughing was done three times per 5-year cycle and ploughing depth was 20 cm. Since 1991, the organically cultivated field had been fertilised with manure, and after 11 years of crop production there was an average positive P balance of 18 kg ha^{-1}. The conventionally cultivated field received mineral fertilisation according to the recommendations of Agri-environmental program of Finland and on average after the 11 year period the P balance was 115 kg ha^{-1} positive. Composite samples were taken in October 2002 from the upper 20 cm soil layers of the plots

representing three management types: unmanaged grassland (grassland), organically cultivated field (organic) and conventionally cultivated field (conventional). They were bulked within management type, sieved (5 mm) and half of the soil representing each management type was air-dried and half was stored field moist at +5°C. The air-dried composite samples were further used for testing the ability of different extraction methods to show land-use -induced changes in soil P (V).

The soil samples for the study on the effect of freezing (III) were taken from the surface horizons of cultivated soils in southern Finland. The clay content in the soils was nearly the same, but they differed in their organic C: one had a C% of 12.8 and is referred to as organic soil and the other had a C% of 1.7 and is referred to as mineral soil. The sampling sites were located approximately 200 m apart on the same field and had practically identical cropping histories. The soils were sampled in the fall and stored at +5°C for 6 months.

The sensitivity of P extraction methods (IV) was studied at the site located in Eastern Finland at MTT Agrifood Research Finland, Maaninka. The samples were taken from two layers (0-2 cm and 2-10 cm) and sieved with a 2-mm sieve and air-dried prior to the analysis.

2.2 Methods and experimental designs

From here on, molybdate-reactive P (MRP) refers to P concentration analysed with molybdenum blue method and is considered to represent the extractable P_i. Molybdate-unreactive P (MUP) is calculated as the difference between the TP and MRP, and represents the extractable P_o.

In the Chang and Jackson fractionation, MRP extracted in each fraction was analysed with a molybdenum blue method modified by Kaila (1955) using stannous chloride as a reducing agent. For the Hedley fractionation, the method of Murphy and Riley (1962) with ascorbic acid as a reducing agent was used. Total P extracted was analysed after acid persulfate digestion ($K_2S_2O_8$; autoclaved for 30 min at 120°C) with molybdenum blue method using ascorbic acid as a reducing agent (APHA, 1989). In both of the fractionation procedures, the strongly coloured NaOH fractions were precipitated with 0.5 M sulfuric acid in acid to sample ratio 1:4 and the clear solutions were analysed for MRP. In NaOH-EDTA extraction the sample was diluted 50 fold prior the analysis of MRP (Murphy and Riley, 1962) and after dilution no precipitation was needed. The analysis of the samples started within in an hour after the addition of colour reagent and the absorbance of the standards were checked in the middle and at the end of the sample measurements.

The P analyses were done with a manually operated spectrophotometer (II, III, V) or with a flow-injection analyser (water-extractable MRP in I and IV). All samples digested for TP determination were analysed with a manually operated spectrophotometer. In paper I, total water-extractable P was analysed with an inductively coupled plasma mass spectrometer (ICP-MS). For each P measurement, the standards were made to have the same matrix as the samples and were treated the same way before the analysis. The methods and procedures used for soil P characterisation are listed in Table 2.

Table 2. Methods used for phosphorus characterisation.

Method	Used in	Extraction ratio	Time h	Centrifugation	Filtration	Measured P forms	Reference
Water extraction							
	III	1:10	21		0.2 µm	TP, MRP, MUP	
	IV	1:50	21		0.4 µm	TP, MRP, MUP	
	I	1:50	21		0.2 µm	TP	
Acid ammonium acetate							Vuorinen & Mäkitie, 1955
0.5 M CH_3COONH_4 +	III	1:20	2		blue ribbon filter	MRP	
0.5 M CH_3COOH, pH 4.65	IV	1:10*	2		blue ribbon filter	MRP	
NaOH-EDTA extraction	V						Turner et al., 2003b
0.25 M NaOH, 0.05 EDTA		1:20	16	30 min 10 000 × *g*	black ribbon filter	TP, MRP, MUP	
Chang and Jackson fractionation	V						Chang & Jackson, 1957
1 M NH_4Cl		1:50	0.5	15 min 1500 × *g*	black ribbon filter	MRP	
0.5 M NH_4F		1:50	1	15 min 1500 × *g*		MRP	
0.1 M NaOH		1:50	16	15 min 1500 × *g*		MRP	
0.25 M H_2SO_4		1:50	1	15 min 1500 × *g*		MRP	
Hedley fractionation	II,V						Hedley et al., 1982
H_2O		1:60	4 + 16	15 min 2600 × *g*	0.2 µm**	TP, MRP, MUP	
0.5 M $NaHCO_3$		1:60	16	15 min 2600 × *g*	0.2 µm**	TP, MRP, MUP	
0.1 M NaOH		1:60	16	15 min 2600 × *g*	0.2 µm**	TP, MRP, MUP	
1.0 M HCl		1:60	16	15 min 2600 × *g*	0.2 µm**	TP, MRP, MUP	

* Volume-based soil to solution ratio

** Half of the supernatant was filtered and MRP was determined only from the fltrate. TP was determined from both, filtered and unfiltered samples.

2.2.1 Pre-treatment -induced changes in phosphorus extractability (I, II, III)

The effects of drying and freezing on soil P solubility were studied with soils from Loppi, Toholampi, Kotkanoja and Sjökulla (I). The samples preserved at sampling moisture or air-dried were frozen (-18°C) or kept at room temperature (ca +20°C) (air-dried) or at +5°C (moist). The solubilities of P, Fe, Al and Mn in the soil samples were studied with water extraction at a soil:solution ratio of 1:50 (mass:volume) (I). Samples were shaken in a centrifuge tube with an orbital shaker for 21 hours and the suspensions were filtered through a 0.2 µm Nuclepore polycarbonate filter. The total dissolved carbon from the water extracts was measured with a TOC analyser. The effect of pre-treatment on the sorption components was studied with acid ammonium oxalate extraction (Niskanen, 1989), which is assumed to extract the poorly-ordered oxides of Al and Fe. The concentrations of Al, Fe and Mn in the oxalate extract and TP in the water extract were measured with ICP-MS. The effects of storage conditions on oxalate- and water-extractable concentrations were analysed for each soil by analysis of variance and pairwise comparisons were done with Tukey's test ($p<0.05$).

The effect of air-drying on more stable P pools was studied with air-dried and field moist samples representing the three management types with decreasing C% (grassland soil, organically cultivated soil and conventionally cultivated soil) (II). Soil P was fractionated using a modified Hedley (Hedley et al., 1982) procedure. In the procedure, 1 g of soil was sequentially extracted with 60 ml of H_2O (16 h + 4 h), 0.5 M $NaHCO_3$ (16 h), 0.1 M NaOH (16 h) and 1.0 M HCl (16 h). The suspensions were centrifuged for 15 min (2600 × *g*) and MRP was determined in the filtered samples (0.2 µm Nuclepore polycarbonate filter) and the TP was determined in the filtered samples and in the centrifuged suspension. In each fraction, MUP, calculated as a difference between TP and MRP, was divided into two size-fractions according to the filter-size (Figure 1). The large-sized MUP (MUP>0.2) was calculated as the difference between TP in the centrifuged sample and TP in the filtered sample (TP<0.2), and small-sized MUP (MUP<0.2) was calculated as the difference between TP<0.2 and MRP. Centrifugation speed and time were adjusted so that, according to the Stokes' equation, the soil particles having a diameter larger than 0.2 µm would settle through the 5-cm high solution column during centrifugation. Particles were assumed to have a density of 2.65 g cm^{-3}. Therefore, the MUP remaining in the solution after centrifugation was assumed to contain mainly low-density organic material, not attached to mineral matrix. The effect of drying on each P faction was analysed by analysis of variance and the pairwise comparisons were done with *t*-test ($p<0.05$).

The freezing-induced changes in the extractability of the slurry and fertiliser P (III) were studied in laboratory incubation experiments with organic and mineral soil untreated or amended with pig slurry or NPK fertiliser. The soils were placed into experimental vessels without drying at any stage. The amount of soil in the vessels was 0.46 dm^3, which corresponded to 500 g of the mineral soil and 308 g of the organic soil on a dry matter basis. One-third of the vessels of both soils were amended with fine-ground commercial

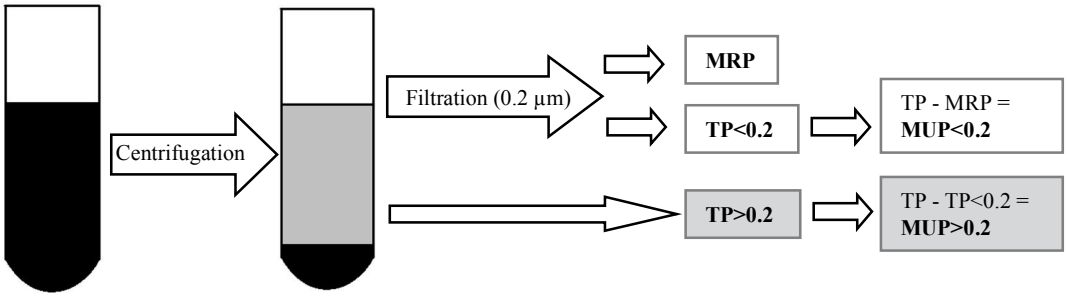

Figure 1. Size-fractionation of extracted phosphorus (II).

compound fertiliser (NPK 20-3-9), one-third with pig slurry, and one-third were incubated with no additions (control). The P addition in fertiliser corresponds to 70 kg ha^{-1} and the addition in the slurry to 94 kg ha^{-1} (in the 20 cm plough layer). Half of the vessels were incubated at a constant temperature of +5°C, and the other half in alternating two-week periods at +5°C and at -20°C (monitored range -17.4 – -22.3°C) for 24 weeks. The water extractions were done at a 1:10 (mass:volume) soil-to-solution ratio, shaken for 21 hours, filtered through a 0.2 µm Nuclepore polycarbonate filter and analysed for MRP and TP. Acid ammonium acetate -extractable P (P_{Ac}), routine advisory method used in Finland for soil testing (STP) was measured as in Vuorinen and Mäkitie (1955): 0.5 M CH_3COONH_4 + 0.5 M CH_3COOH, pH 4.65; 1:20 w:v, 1 h shaking. The P_{Ac} was analysed only for MRP. The effect of freezing on P solubility was tested by analysis of variance and pairwise comparisons were done with Tukey's test (p<0.05).

2.2.2 Sensitivity of phosphorus tests in monitoring changes in phosphorus solubility (IV)

The sensitivity of two routinely used extraction tests (water extraction and acid ammonium acetate extraction (Ac)) to detect changes in the soil P status was studied in a field experiment on pasture land with three treatments: control, urine addition and dung addition (IV). The field was divided into three blocks and on each block a total of ten cattle dung, cattle urine or control patches were randomised. The excreta was mixed, sampled to be applied to the field an hour after collection. Portion of dung (2.47 kg) that represented an average-sized dung patch was spread on round plastic netting (Ø 36 cm) and laid on the grass stubble on the field. The weighed urine (2.37 kg) was spread evenly onto the field within the border of a collar having a size corresponding to the area of a single urination (Ø 67 cm). Soil samples from the urine and control patches were taken 0, 1, 3, 5, 10, 21, 49, 77 and 120 days after the beginning of the experiment. The sampling of soil below the dung patches began on day three and continued as above. The sampling depths were 0-2 cm and 2-10 cm. The samples were left to dry at temperature (ca +20°C) and the analysis was carried out with the air-dried soil.

The water extractions were done at a 1:50 (IV) (mass:volume) soil-to-solution ratio, shaken for 21 hours, filtered through a 0.4 µm (IV) Nuclepore polycarbonate filter and analysed for MRP and TP. Acid ammonium acetate -extractable P (P_{Ac}) was measured as

in Vuorinen and Mäkitie (1955): 0.5 M CH_3COONH_4 + 0.5 M CH_3COOH, pH 4.65; 1:10 v:v, 1 h shaking. The suspensions were centrifuged and analysed for MRP with an autoanalyser. To make the comparisons of the sensitivity of water extraction and STP (P_{Ac}) for reflecting dung- or urine-induced changes easier, the bulk density of the air-dried soil was used to correct the per volume results of STP to per kilogram of soil. The effect of urine and dung additions on water-extractable and acid ammonium acetate -extractable P was tested by analysis of variance. Dunnett's test ($p<0.05$) was used to compare the treatment-induced change in P solubility compared to the control sample.

2.2.3 Comparing the characteristics revealed by different extraction methods (V)

The ability of different extraction methods to reveal changes in soil P pools was tested with soils representing three management types (grassland soil, organically cultivated soil and conventionally cultivated soil) (V). The P_i was fractionated using the Chang and Jackson (1957) procedure modified by Hartikainen (1979). One gram of soil was sequentially extracted with 50 ml of 1 M NH_4Cl (30 min), 0.5 M NH_4F (1 h), 0.1 M NaOH (16 h) and 0.25 M H_2SO_4 (1 h). The suspensions were centrifuged for 15 min (1500 × *g*). Between the three last extractions the soil was washed with a saturated solution of NaCl. The extracts were analysed for MRP. In addition to MRP, the modified Hedley (Hedley et al., 1982) procedure described in section 2.2.1 was used to characterise soil MUP. Total extractable P was calculated as the sum of TP in all fractions. For these composite soil samples, the analysis of variance was done for each P fraction to calculate the standard errors (SE).

The NaOH-EDTA-extractable P_o was measured after a 16-hour extraction (10 g soil to 200 ml of NaOH-EDTA -solution (0.25 M NaOH + 0.05 M EDTA). The suspension was centrifuged (30 minutes 10 000 × *g*) and the supernatant was filtered (black ribbon) to remove detritus. The filtrate analysed for MRP and for TP and the concentration of MUP was calculated as the difference between them. The NaOH-EDTA-extractable P was further characterised with solution ^{31}P-NMR spectroscopy. The NaOH-EDTA extracts were freeze-dried (-20^{o}C) and one gram of freeze-dried sample was dissolved into 0.4 ml of NaOH-EDTA solution (1 M NaOH, 0.1 M EDTA) and 0.5 ml of deuterium oxide and 2.1 ml of water were added. The pH of the samples was adjusted to be >13 and after centrifugation (1500 × *g*) samples were decanted into 10 mm ^{31}P-NMR-tubes and analysed with a Varian Inova 500 spectrometer.

3 Results and discussion

3.1 Sampling the surface soil from agricultural fields

3.1.1 Sampling depth

In arable fields ploughed annually, the regular mixing of surface soil is expected to produce a relatively homogenous soil layer. However, on farm land under no-tillage, on pasture land and in buffer strips P has been found to enrich in the uppermost surface layer (e.g. Rasmussen, 1999; Jansson & Tuhkanen, 2003; Muukkonen et al., 2007). The different stratification of soil P in differently managed soils complicates sampling. In field study (IV), the dung and urine induced-increase in water-extractable MRP and P_{Ac} concentrations was seen only in the uppermost surface soil (0-2 cm). In these cases, sampling at too great a depth can result in errors caused by mixing P-enriched topsoil with a larger volume of soil (Turtola & Yli-Halla, 1999). In experiments as described in papers II and V, where the three differently managed soils representing unmanaged grassland and organically and conventionally cultivated fields were sampled to the same depth, some management-induced differences may have been obscured. In the grassland soil, the uppermost 1 cm, most likely being enriched by organic C and P, was being mixed with a larger volume of soil causing dilution and possibly enabling transformation of P from one pool to another through sorption reactions with newly revealed surfaces. Thus, the different stratification of P in different kind of land use types highlight the importance of carefully planned sampling. To get representative samples for the purpose intended, the land-use history should be studied beforehand and taken into account in sampling.

3.1.2 Time of sampling

In the published literature, evidence on the importance of sampling time to the interpretation of STP results is contradictory. Kuo and Jellum (1987) recommended on the basis of their field study that the time of sampling should be carefully considered when assessing P availability in soil, whereas Read and Cameron (1979) and Ball and Williams (1968) found only small differences in the P availability between fall and spring samples.

In Finland, surface soils are frozen at least some time during the winter and almost always several freeze-thaw cycles occur during the freezing period. In many laboratory experiments freezing has been shown to increase the soluble P concentration in soils (Hinman, 1970; Ron Vaz et al., 1994; Freppaz et al., 2007), which leads to the assumption that analyses of STP are affected by sampling time. In contrast, the laboratory incubation study presented in paper III did not show soil freeze-thaw cycles to affect the P_{Ac} or water-extractable MRP or MUP, thus suggesting that the sampling time (fall vs. spring) would not have a significant effect on STP results. However, in these soils, the relatively high

STP could have masked the freezing-induced differences. On the other hand, soils that freeze yearly may become adapted to freezing, suggesting that more dramatic changes would be expected in soils not exposed to below-zero conditions regularly. Furthermore, the incubation study (III) was done without any plants. Räty et al. (2009), who found the P concentration in the dying plant material to decrease after the first frosts, proposed that the seasonal changes in soil P solubility may be explained by decaying plant material entering the soil. The findings of Shand et al. (1994) showing that the presence of plants influenced the amount and forms of P in soil solution further highlights the importance of plants in soil P cycling. Thus, laboratory incubation studies done without plants reveal only the changes taking place in soil. Therefore, the incubation studies, in which the yearly cycle in plant growth is not taken into account, do not reveal the natural cycling of P and thus, the results can not be directly applied to field conditions.

During the growing season, P is affected by plant growth and biological activity which, in turn, is controlled by meteorologically influenced soil conditions (Tate et al., 1991; Chen et al., 2003). In paper IV, P_{Ac} showed a decreasing trend during the growing season which is in line with the findings of Kuo and Jellum (1987), Tabaglio et al. (2006) and Styles and Coxon (2007) who found the STP to decrease in the summer as a result of plant P uptake. Obviously, for cultivated soils, another factor affecting P pools during the growing season is fertilisation. Even if P is not included in the applied fertiliser, the effect of fertiliser addition on biological activity and, through increased salt concentration, on sorption reactions leads to changes in the biogeochemical cycle of P. The water-extractable MRP showed a large decrease right after the NK-fertilisation, whereas no change was detectable in the P_{Ac} (Figure 2, IV). Kuo and Jellum (1987) found the seasonal variations in water-extractable MRP to be pH dependent and because soil pH was negatively correlated with NO_3-N and NH_4-N and with temperature they suggested that the seasonal changes in water-extractable MRP were at least partly regulated biologically. In our study, the increase in MUP during the growing season (Figure 2) also suggests that water-extractable MUP represents a P pool that is of biological origin. Similarly, Styles and Coxon (2007) reported water-extractable MUP to increase through spring into summer.

These findings illustrate, that the extraction methods have different sensitivities for showing seasonal and management-induced changes in the P pools. Thus, the importance of sampling time on the result interpretation should be considered separately for each method. Since P forms solubilised with different extractants appear to show dissimilar seasonal variation, this information could be combined to increase our knowledge on the role of extracted P forms in the dynamic P cycle and further, to form a profound conception of soil P cycling in different ecosystems.

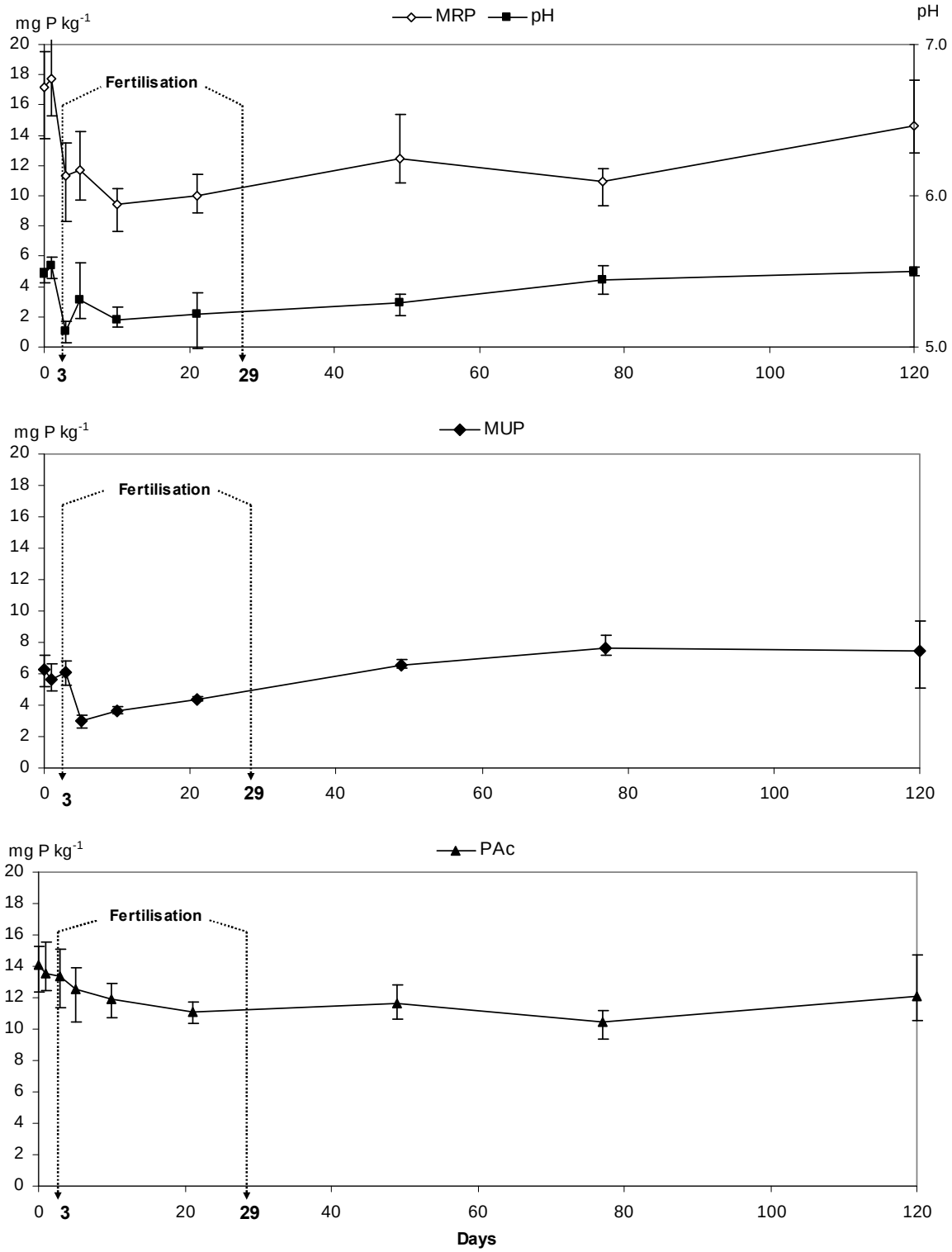

Figure 2. Changes in the pH and water-extractable molybdate-reactive P (MRP), molybdate-unreactive P (MUP) and acid ammonium acetate-extractable P (P_{Ac}) in the uppermost surface soil (0-2 cm) between the beginning of June and the end of September in 2003 (IV). NK-fertilisation was done on the third and 29th days. Error bars represent the highest and lowest value of the three replicates.

3.2 Pre-treatment -induced changes in extractable phosphorus

To get results representing the soil P concentration in the field at the time of sampling, the analysis should be conducted as soon as possible. In practice, this is not possible in advisory soil testing and usually the samples need to be stored before the analysis. The most economical way to store samples is to air-dry them. However, air-drying affects P solubility (I; II; Rubæk & Sibbesen, 1993; Turner & Haygarth, 2001; Turner & Haygarth, 2003; Styles & Coxon, 2006) as well as sorption components (I; Haynes & Swift, 1985). These results, showing increased sorption and at the same time increased solubility, can be explained by the structural changes in soil organic matter and organo-mineral complexes.

Styles and Coxon (2006) and Turner and Haygarth (2001) found that MUP accounted for two-thirds of the drying-induced increase in total water-extractable P, suggesting that soil P_o is more prone to changes due to drying than P_i. The death of soil microbes or cell lysis due to drying and rewetting is most likely one source of increased labile P concentrations in soils (Turner et al., 2003b). In our study (II), the drying-induced increase was significant in the water-extractable P analysed from the filtered samples, but when P was analysed from centrifuged but not filtered samples the drying-induced change was much smaller, and was not statistically significant (Figure 3, II). This supports the theory regarding structural changes in the organic matter and suggests that the increase in MRP and small-sized MUP in the filtered samples could partly originate from the large-sized MUP that is left out of the analysis in the filtering procedure.

Most studies looking into effects of air-drying on soil P have concentrated on labile pools and on soil test P (STP). Schlichting and Leinweber (2002) did the Hedley fractionation for peat soil and found air-drying to decrease P solubility in all fractions, suggesting that the role of more stable P forms should not be neglected. We fractionated three differently-managed clay soils according to the Hedley procedure (II) and air-drying did not change the total amount of P extracted but did increase the small-sized MUP (MUP<0.2) concentration in NaOH fraction. However, as the NaOH-extractable small-sized MUP increased, the large-sized MUP decreased, suggesting that the increase in small-size MUP originated from the break-up of large-sized compounds. According to Schlichting and Leinweber (2002), in peat soil the drying-induced decrease in solubility of less labile P forms was explained by irreversible shrinkage of organic matter and inclusion of otherwise extractable P. However, in minerals soils the drying-induced changes in the more stable P fractions are most likely due to the structural break-up of organic matter and organo-mineral interactions (II).

In contrast to air-drying, freezing of samples and storage at temperatures below zero was found not to have significant effects on soil P solubility or the Fe- and Al-oxides that act as P sorption surfaces (I). Ron Vaz et al. (1994) reported the after-freezing increase in P solubility to be largest in soil having a high organic matter concentration. Thus, the lack of freezing-induced responses in the mineral soils studied in paper I may be explained by the lower organic matter concentration. However, in the laboratory incubation study done with organic soil, no response to freezing was detected (III), but in here, the relatively high STP of the soils may have masked the changes.

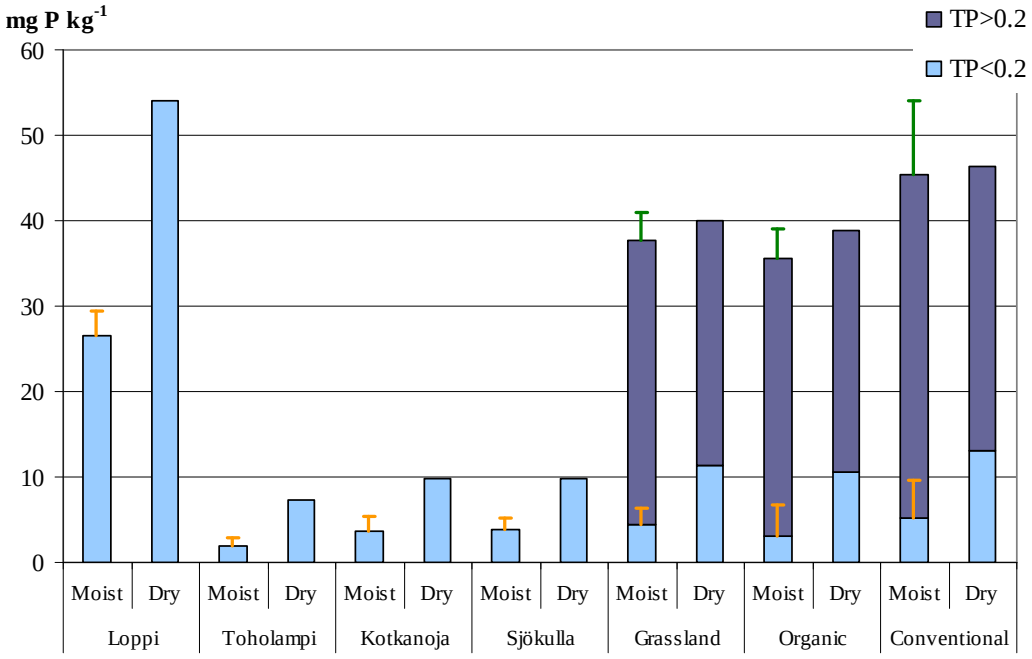

Figure 3. Water-extractable P in moist and air-dried samples (I, II). TP<0.2 is total P analysed from filtered (membrane filter, pore size 0.2 µm) samples and TP>0.2 is total P analysed from centrifuged unfiltered samples. The effect of air-drying on soil P solubility was tested within each soil with analysis of variance. Orange error bars represent least significant difference (LSD) (t-test p<0.05) for TP<0.2 and green error bars represent the LSD (t-test p<0.05) for total water-extractable P in centrifuged samples.

Freezing has been suggested to resemble drying because the formation of ice leads to a decrease in liquid water content in the soil. The differences in the P solubility in samples stored air-dried or frozen (I) may originate from the rapidity of the remoistening. Rewetting of air-dried soil rapidly changes the osmotic potential in the soil and the osmotic shock can lead the cells to excrete intracellular solutes to maintain the balance, or to the disruption of microbial cells (Halverson et al., 2000). However, melting is a slower process, allowing a longer time for adaptation, and thus, the consequences are not so drastic. On the other hand, the soils in Finland are subjected to multiple freeze-thaw cycles yearly and the microbial community can be expected to be adapted to these cycles. Kieft et al. (1987) hypothesised that the response to changes in water potential is lower in soil having a larger amount of microbes which can, with the help of organic intracellular solutes, rapidly modulate their internal water potential.

Drying has been suggested to boost the nutrient cycle (see Kieft et al., 1987) and Nguyen and Marschner (2005) highlighted the importance of dry-wet cycles for increasing the cycling of P from native organic matter. Even though the drying of soil in the field is not as uniform as air-drying taking place in the laboratory, the drying-induced increase in soil P solubility (I, II) might represent the proportion P that is in continuous biological cycle and indicate the potentially plant available P pool (II). Thus, when the purpose of

sampling is to estimate the potential of soil to release bio-available P, soil samples can be air-dried prior to the analysis. However, soil samples should not be dried when studying the solubility of P in the field or dynamic cycling of P in the nature. Especially in the boreal zone, instead of drying, the samples can be stored frozen if the analyses cannot be conducted soon after sampling.

3.3 Effect of extractant and sample preparation procedure on the extractable phosphorus

3.3.1 Buffered solutions as extractants

In Finland, soil testing is done using an acid ammonium acetate extraction (Ac) buffered to pH 4.65. Its use is based on a large number of field experiments and it has been found to provide a good estimation of plant-available P in acidic Finnish soils (Saarela, 2002). When the ability of this buffered solution to detect changes in the P concentration was compared to water extraction, we noticed a single dung addition to result in an increase in both P_{Ac} and MRP in the upper 0-2 cm soil layer (IV). This result shows that an increase in soil P concentration can be detected with both methods and supports the finding of Jansson and Tuhkanen (2003) that P_{Ac} can be used to give an estimate for the P loading potential of pasture. However, the known increase in P solubility after urea addition (Hartikainen & Yli-Halla, 1996; Shand et al., 2000) was detected only in the water-extractable MRP, whereas the P_{Ac} did not show the urine- or fertilisation-induced changes in P solubility (Figure 2, IV). The difference between the outcomes is attributable to the fact that the buffered Ac extracts soil at a low pH (4.65), enhancing sorption of P, whereas in the water extraction the P extracted depends on the actual pH in the soil. These results suggest that the sensitivity of the Ac test to react to changes in P chemistry due to alterations in the external conditions is relatively poor, whereas water extraction is more sensitive to environmental changes. Thus, the use of buffered solution obscures changes in soil P solubility that might be of environmental importance. However, the sensitivity of water extraction, which is an advantage when studying the environmental changes affecting P solubility, can be a disadvantage in agronomic soil testing and may even lead to erroneous conclusions. For example, the urine-induced increase in soil pH and the subsequent increase in water-extractable MRP would have lead to overestimation of long-term plant-availability of P, since the effect of urine diminished after ten days (IV).

3.3.2 Effect of ionic strength of the extractant

An increase in the ionic strength of soil solution increases P sorption (Ryden & Syers, 1975) and on the other hand, decreases desorption (Barrow, 1979a). In paper V, the MRP extracted with water was larger than the MRP extracted with NH_4Cl, reflecting the

dependence of P desorption on ionic strength (V) and supporting the findings of Hartikainen and Yli-Halla (1982) showing that extraction with dilute extractants enhances the P desorption.

The extractable MUP concentration appeared to be affected by the ionic strength of the extractant (II). When H_2O- and $NaHCO_3$-extractable MUP fractions were divided into two size-classes by filtering the centrifuged extracts through a membrane filter (procedure showed in Figure 1), $NaHCO_3$, having a higher salt concentration, released hardly any large-sized MUP whereas in water-extractable P, the larger-sized MUP constituted the major part of the total extractable P (Figure 4, II). According to Koopmans et al. (2005), the low ionic strength of a high soil-to-solution ratio water extraction leads to soil dispersion and detachment of colloids from soil and on the other hand, the increase in ionic strength leads to flocculation of colloidal material. Despite the high ionic strength, NaOH extraction like water extraction produced high amounts of larger-sized MUP (II). However, in the high pH of NaOH many organic functional groups are ionized and the increased charge density leads to increased solubility of organic molecules (Swift, 1996). Further, the instability of organic molecules also increases as sodium takes the place of polyvalent cations, causing swelling and dispersal of clays and organic material (Oades, 1988; Swift, 1996). Thus, NaOH extraction changes the physical structure of organic molecules in a way that enhances their solubility.

Based on the results demonstrating a role of P_o in plant nutrition (Sharpley, 1985; Stewart & Tiessen, 1987), including analysis of P_o in the routine soil tests has been suggested (McDowell & Koopmans, 2006). The variation of extractants used in plant-availability estimations is large suggesting that there are differences in their ability to solubilise organic matter. Thus, the suitability of an extractant, originally developed for extracting plant-available P_i, should be carefully tested for its ability to extract the pool of P_o actually contributing to plant-available P.

3.3.3 Centrifuging and filtering the suspensions

Filtration is important in the MRP analysis because colloidal particles can disturb the colorimetric analysis. The disturbance can originate from turbidity affecting the absorption of light during the analysis (Koopmans et al., 2005), but also solubilisation of colloid-bound P by the acid molybdate reagent can contribute to the increase in the MRP concentration (Hens & Merckx, 2002). On the other hand, in the acidic conditions required for the colour formation, P associated with organic matter may precipitate leading to the underestimation of MRP in solution (Turner et al., 2006). However, although filtration of samples for MRP analysis can be recommended, the filtration left a large proportion of water- and NaOH-extractable MUP (MUP >0.2 µm) out of the measurement (Figure 3, II). Previously, Gimbert et al. (2005) found filtration to remove a significant proportion of the colloidal fraction and suggested that centrifugation should be preferred when studying colloidal material in soil suspensions. However, the results showing that hardly any large-sized MUP was released during $NaHCO_3$ extraction (Figure 4, II) suggest that centrifugation of high ionic strength suspensions resulted in the collapse of larger

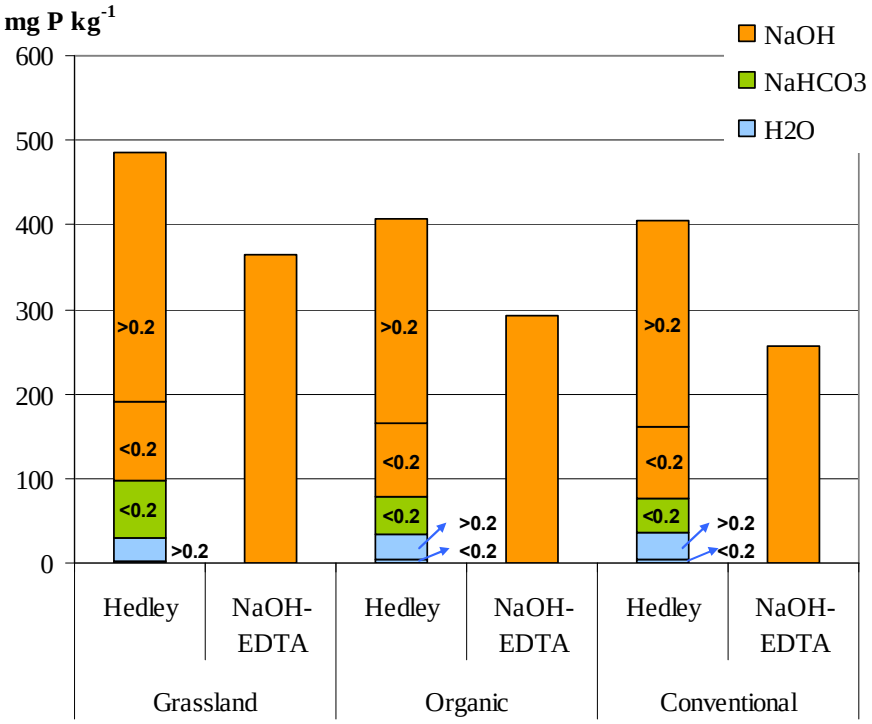

Figure 4. Molybdate-unreactive P (MUP) in unmanaged grassland and in organically and conventionally cultivated soils extracted with the Hedley fractionation procedure and with NaOH-EDTA. In the Hedley procedure each fraction was divided into two size-fractions by filtering the centrifuged samples through a 0.2 µm membrane filter (II, V).

molecules and the subsequent flocculation with clay particles resulted in their removal from the suspension (II). In fact, Poile et al. (1990) found Olsen P to diminish with prolonged centrifugation time and the reduction was associated with a decline in organic carbon in the $NaHCO_3$ extract.

The sensitivity of MRP and MUP analyses to filtering and extractant properties are especially critical in the sequential fractionation procedures, for it is essential that all solubilised P is analysed in each fractionation step and that no sample is lost during the procedure. Filtration, which removes the colloids that can disturb MRP analysis, complicates routinely done sequential extractions because the filter and the sample retained on it should be included in the subsequent extractions. The need for filtration can be overcome with high speed centrifugation (Gimbert et al., 2005); however, the ionic strength of the extractant, which has a strong influence on the settling of particles during centrifugation (II), varies in each step of the sequential fractionation procedures. Thus, the use of filtration is questionable when estimating the concentration of extractable MUP, but the sensitivity of MUP to the sample preparation procedure in extractants with high salt concentration should be kept in mind when comparing published results. These findings highlight the importance of detailed characterisation of colloidal P solubilised in extractions as well as characterisation of the MUP fraction that is removed during the centrifugation.

3.4 Extraction methods in identification of chemical form of phosphorus

3.4.1 Characterisation of inorganic phosphorus

Clayey soil that had been managed differently for 11 years (grassland soil, organically cultivated soil and conventionally cultivated soil) was used to study the characteristics of soil P_i revealed by the Chang and Jackson and Hedley sequential fractionation procedures and NaOH-EDTA extraction (V). Both of the widely used fractionation procedures showed an increase in soil MRP in cultivated soils receiving fertilisation compared to unfertilised grassland soil (Figure 5). In the Chang and Jackson fractionation procedure, increases were seen in NH_4F- and NaOH-extractable MRP, reflecting P bound to Al- and Fe-oxides being highest in soil having the most positive P balance (V). This outcome is in agreement with earlier studies (Kaila, 1964a; 1964b; Sharpley & Smith, 1985) showing that the addition of P fertiliser increases the P sorbed onto Al- and Fe-oxides. As for fractions from the Hedley procedure, $NaHCO_3$- and NaOH-extractable MRP represented a similar sink for P applied in excess of plant uptake (Figure 5, V), which agrees with results of Schmidt et al. (1996).

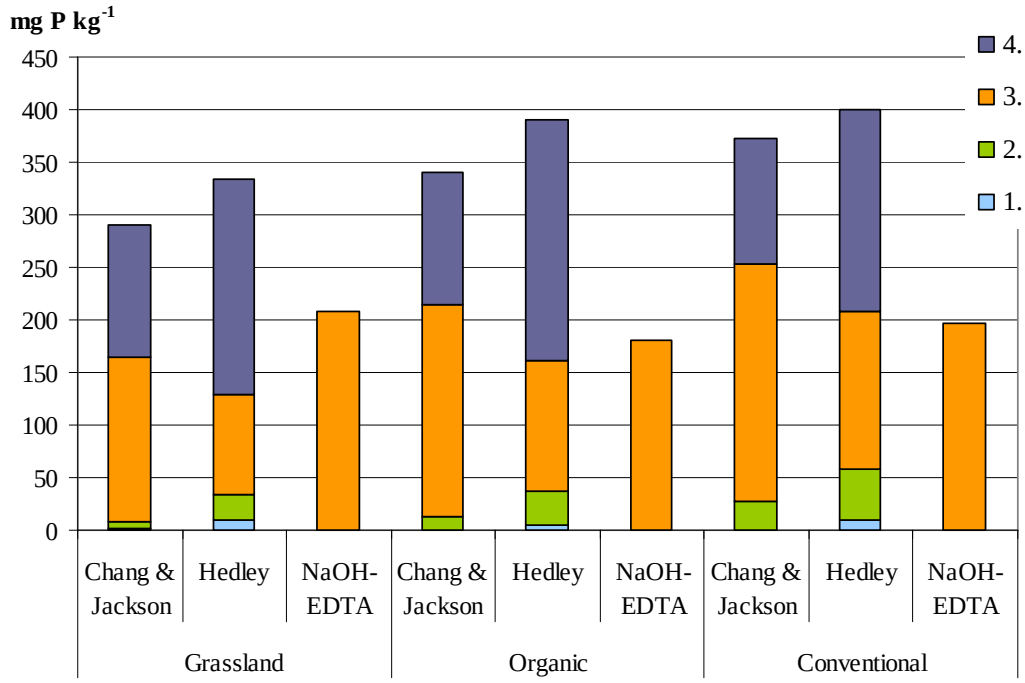

Figure 5 . Molybdat e-reactive P (MRP) from un managed gra ssland soi l and in org anically and conventionally c ultivated soils extracted with Chang and Jackson and Hedley sequential fractionation procedures and with NaOH-EDTA (V). Colours refer to fractionation steps that were in the Chang and Jackson procedure ***1.*** *NH_4Cl;* ***2.*** *NH_4F;* ***3.*** *NaOH;* ***4.*** *H_2SO_4 a nd in t he Hedley procedure* ***1.*** *H_2O;* ***2.*** *$NaHCO_3$;* ***3.*** *NaOH;* ***4.*** *HCl.*

The two first fractionation steps (H_2O + $NaHCO_3$) in the Hedley procedure extracted more MRP for these differently managed soils than the two first Chang and Jackson extractions (Figure 5, V). This is in line with the shorter extraction time in the NH_4F extraction and with the selectivity of NH_4F to solubilise only Al-bound P, whereas $NaHCO_3$ extracts weakly bound P from all sorption surfaces. Also readsorption of NH_4F-extractable P during the extraction has been reported (Rajendran & Sutton, 1970). In both procedures, the next extraction is done with NaOH, and as both procedures have analogous extraction steps, the sum of solubilised MRP after comparable steps should theoretically be equal. However, in the Hedley procedure, the sum of MRP after the NaOH extraction was lower than that in the Chang and Jackson procedure, because of the lower NaOH-extractable P (Figure 5, V). It is possible that in the Hedley procedure, the solubilisation of P was incomplete or readsorption of desorbed P took place during the extraction. It can be assumed that in the Chang and Jackson procedure, the readsorption of NaOH-extractable P onto Al oxides was less probable because of the stable complexes formed between Al and F in the preceding extraction step. Further, in the Hedley procedure, the second extraction step done with $NaHCO_3$ extracted more P than the second step in the Chang and Jackson procedure. As a consequence of decreased P concentration on the sorption surfaces the strength of bonds between P and Al or Fe oxides may have increased affecting the ability of NaOH to extract P. In the Chang and Jackson procedure, the NH_4F extraction preceding NaOH extraction extracted less P than the second extraction step in the Hedley procedure and as the concentration of P was larger on the sorption surfaces during the NaOH extraction in the Chang and Jackson procedure the weaker bonds between sorption surfaces and P may have increased the extractability of P in NaOH. These results demonstrate that the extractability of P in a given step is affected by the preceding steps. This hypothesis was supported by the finding that the acid extraction step following the NaOH-extraction in both procedures resulted in higher MRP for the Hedley procedure than for the Chang and Jackson procedure (Figure 5, V). Actually, He et al. (2006) found that the acid extraction step in the Hedley procedure extracted Al- and Fe-bound P.

In contrast to the fractionation procedures, a single NaOH-EDTA extraction, used to extract P for ^{31}P-NMR analysis, did not reveal the increasing trend in MRP with increase in positive P balance (Figure 5, V). This suggests that NaOH-EDTA extracted P from different pools or with different mechanisms than NaOH extraction step in the fractionation procedures. For the grassland soil having highest organic matter content, the MRP extracted with NaOH-EDTA was higher than that recovered with the three first extraction steps in the fractionation procedures. The effectiveness of NaOH-EDTA extraction in solubilising MRP may be due to the breaking down of cationic bridges between organic matter and PO_4-P whereas in the Hedley procedure, the alkali-extractable organically complexed MRP might end up as a part of MUP because of the precipitation of organic matter in the acidic conditions required for the colour development in the colorimetric analysis.

In other studies, colorimetric determination of NaOH-EDTA-extractable P has resulted in lower MRP concentrations compared to PO_4-P calculated from the ^{31}P-NMR spectra (Turner et al., 2003c; Turner et al., 2006). On the contrary in here, for the three differently

managed clayey surface soils the colorimetrically analysed MRP concentration was higher than that of calculated from the ^{31}P-NMR spectra (V). This difference could be explained with acid hydrolysis of P_o compounds taking place in the acidic condition during the colour formation (Turner et al., 2005). However, because the difference between the two methods was highest in the soil having lowest P_o concentration and, on the other hand, highest P_i concentration the role of colloidal particles releasing MRP in the acidic conditions can not be ruled out (V).

The comparison of the characterisation methods revealed that in sequential extractions, extractability of P is affected by preceding steps (V). Rubæk and Sibbesen (1995) suggested also that during sequential extractions redistribution of P_i and P_o may take place. Based on the literature, the analysis of extracted P_i with molybdenum blue method in prone to errors that may originate from precipitation of organic matter and P_i occluded within the organic structures (Turner et al., 2006), from disintegration of complexes where P is bound to organic molecules through cationic bridge (Gerke & Jungk, 1991) or from hydrolysis of P_o compounds (Turner et al., 2005). Despite the uncertainties of operational P fractionation the advantage of sequential extractions, compared to single NaOH-EDTA extraction and ^{31}P-NMR spectroscopy, lays on studying treatment-induced changes in P pools with different solubility.

3.4.2 Characterisation of organic phosphorus

The lower organic C% in conventionally and organically cultivated soils compared to grassland soil (Table 1) was reflected as decreased MUP concentrations, calculated as a sum of all Hedley fractions (Figure 4, II, V). Similar cultivation-induced changes in soil P_o have been reported in several studies (Schollenberger, 1920; Haas et al., 1961; Tiessen et al., 1982; Sharpley & Smith, 1985; Bowman et al., 1990; Condron et al., 1990). The NaOH-EDTA extraction revealed a trend of decreasing MUP in the following order: grassland soil > organically cultivated soil > conventionally cultivated soil (Figure 4, V). This trend being in line with a decrease in soil C% supports the conclusion of Guggenberger et al. (1996) that the alkali-extractable MUP reflects the overall changes in soil P_o due to cultivation.

For all soils representing the three different management types, NaOH-EDTA extracted less MUP than the Hedley procedure (Figure 4, V). In the latter, no MUP was recovered in the last acid extraction and thus, the total MUP represented the sum of the three first fractions. The difference between the two methods can be speculated to originate from differences in extraction and sample preparation procedure (preceding extractions and differences centrifugation speed) or from the different mechanisms in solubilising P_o. The difference in MUP between the two methods decreased with increasing organic C% in the samples (V), supporting the findings of Bowman and Moir (1993) who concluded that the NaOH-EDTA extraction is effective in soils high in organic matter where the chelation with metal cations plays an important role in tying up P_o.

Despite the different concentrations recovered with the Hedley's $NaHCO_3$ and NaOH extractions for the differently managed soils, the ^{31}P-NMR analysis resulted in fairly similar P_o composition in these three differently managed soils; the proportions of various P_o compounds of the total NaOH-EDTA-extractable P_o were only slightly affected by the change in land use (V). Similarly, the relative distribution of MUP between Hedley's labile (24-25%) and moderately labile NaOH-extractable (75-76%) fractions was not affected by land use, suggesting that at least in short term all fractions contributed similarly to the land-use -induced decrease in the P_o. In contrast, Condron et al. (1990) found that soils, cultivated over 70 years, contained only monoesters providing evidence that the less stabile forms of P_o had been preferentially degraded. In our study, all three differently managed soils contained small proportions of diesters, suggesting that the relatively short time (11 years), when the soils had been managed differently, has not brought out such differences in P_o quality that could be detected after the long sample preparation procedure required for ^{31}P-NMR analysis. These results demonstrate that the extraction methods were able to show the differences in P_o concentrations of soils with decreasing C%. However, with these soils $NaHCO_3$-extractable MUP suggested to represent labile P_o could not be demonstrated to be more easily mineralised than the NaOH-extractable MUP. Taking account the large heterogeneity of P_o, it is unlikely that the sequentially extracted P_o fractions would represent a certain functionally similar group, but it is more likely that specific groups are present in more than one fraction (Turner et al., 2005).

3.5 Practical applicability of chemical extraction data

The STP values obtained with the acid ammonium acetate (P_{Ac}) test and the Hedley's labile MRP (sum of H_2O- and $NaHCO_3$-extractable MRP) showed similarly that plant-available P_i was higher in cultivated soils receiving fertilisation (II, V). Increases in P_{Ac} and Hedley labile MRP can be explained by the P balance increasing in the same order. However, when taking account the labile MUP, the total labile P (MRP + MUP) in differently managed soils end up being nearly the same in the high and low STP soils (V). Sharpley (1985) found that in unfertilised soils the available P was closely related to P_o content, whereas in fertilised soils there was a close relationship between P_i and available P. Similar findings suggesting that the role of P_o in providing available P for plant production is highlighted in soils low in plant available P_i have been published by Guo et al. (2000), Buehler et al. (2002) and Styles and Coxon (2006). This suggests that the biogeochemical cycle of P varies in differently managed soil. This dissimilar role of P_o in providing plant-available P in different kind of ecosystems could also partly explain the contradictory results on the plant-availability of bicarbonate-extractable MUP reported in the literature (see Bowman & Cole, 1978; Hayes et al., 2000). Thus, if the extracted MUP that is thought to represent the labile P_o may have a dissimilar function in differently managed soils, the estimation of the amount of plant-available P with extraction methods will be challenging. For interpretation of the results the function of extracted P pool in the P cycle should be known in the system under investigation.

Repeated fertiliser additions result in accumulation of P in the sorption surfaces and this accumulation can be quantified by the calculation of saturation index. For acid soils, saturation index has been suggested to be calculated as a ratio of Chang and Jackson's NH_4F- and NaOH-extractable MRP (P sorbed onto Al- and Fe-oxides) to the shortrange ordered Al- and Fe-oxides extracted with acid ammonium oxalate (Peltovuori et al., 2002). Because the P on sorption surfaces and in the soil solution tend to be in equilibrium, the higher P saturation results in higher P concentrations in soil solution and higher plant availability. In our study, with differently managed soils of the same geological origin, the Chang and Jackson fractionation procedure showed that the Al- and Fe-bound MRP was lower in grassland soil compared to organically and conventionally cultivated soils receiving P fertilisation (V). This suggests that the P saturation of sorption surfaces is higher in cultivated soils. However, the unmanaged grassland soil, which had the lowest concentration of P bound to Fe- and Al-oxides, had according to Hedley procedure, the highest MUP concentration (V). Taking into account that the inositol phosphates are retained by ligand exchange to the same sites as orthophosphate (Anderson & Arlidge, 1962; Celi et al., 1999) this suggest that the P saturation of the sorption sites may be underestimated by measuring only MRP. However, because of the large heterogeneity of P_o and their sorption ability, measuring only total P_o may not give additional value to the estimation of surface saturation but instead; the more detailed characterisation of P_o would be required for improved surface saturation estimates.

All widely used extraction methods are products of an intensive research in which they have been found to be suitable for the those purposes they have been developed for. However, there is a wide diversity of extractants that aim to solubilise the same functional P pool but give different results. The methods discussed in this thesis, showed to be suitable for differentiating soils according to the extractable P concentration, but however, the results showed that in addition to the effect of the used extractant, the outcome was sensitive for sample preparation and extraction procedure. This complicates the comparison of the results between different studies. The comparisons and furthermore, the interpretations of the results, are complicated even more by the fact that the P cycling is likely to differ between land-use types and management types and the extracted P form may have a dissimilar function in different situations.

3.6 Conclusions

The use and management of agricultural fields affect the horizontal and vertical distribution of soil P. This distribution, together with natural spatial variability, complicates soil sampling. However, an understanding of land-use history and the purpose of collecting analytical data can facilitate the design of an effective sampling strategy.

Various laboratory procedures result in alterations in soil P reserves. Soil air-drying was found to change the solubility of P. The simultaneous increase in sorption affinity and P solubility can be explained by structural alterations in soil organic matter leading to the exposure of new sorption surfaces. Structural disruption was further evidenced by an increase in the solubility of water-extractable, small-sized (<0.2 µm) P that originated, at

least partly, from the pool of large-sized (>0.2 µm) P. Freezing was found to have an insignificant effect on soil P for Finnish soils; thus, samples that require storage prior to analysis should preferably be stored at below zero temperatures, rather than air-dried.

In addition to the effects of sample pre-treatment, the results showed that molybdate-unreactive phosphorus (MUP), thought to represent organic P, was sensitive to the chemical nature of the used extractant and to the sample preparation procedures employed prior to P analysis, including centrifugation and filtering of soil suspensions. Filtering may remove a major proportion of extractable MUP; therefore filtering cannot be recommended in the characterisation of solubilised MUP. However, extractants having high ionic strength may cause the organic molecules to collapse during centrifugation and thus affect the recovered concentration of MUP. These findings highlight the importance of characterising the nature of the MUP extracted with different extractants and acknowledging the sensitivity of MUP to analytical procedures when comparing published results.

The chemical nature of the extractant affects the ability of the method to detect changes in the solubility of soil P that are not related to the total concentration of P in the soil. Water extraction was found to be sensitive to physicochemical changes in soil solutions, and can be recommended for estimating short-term changes in P solubility. However, this sensitivity can be a disadvantage in agronomic soil testing that aims to describe soil P status over the longer term. The sensitivity of the method used to detect changes in P solubility plays a key role in the interpretation of results obtained at different times of the year.

A comparison of the two fractionation methods studied here, Chang and Jackson fractionation and Hedley fractionation, revealed that in sequential extraction procedures, the preceding extractions affected the solubility of P in the remaining fractionation steps. Therefore, chemically extracted P fractions do not correspond to the natural distribution of soil P. For unmanaged grassland soil that was low in soil test P (STP) and in Hedley's labile molybdate-reactive P (MRP, thought to represent the inorganic P), the total labile P was similar to that found in fertilised soils due to the high concentration of labile MUP. This highlights the need for a detailed characterisation of the plant availability of MUP. The results of this study demonstrate that, although the extraction methods do not reveal the biogeochemical function of a given P pool in soil, the extraction methods can be used to detect changes in soil P pools with different solubilities. To obtain the most benefit from extraction methods, we need a better understanding of the biological availability of P and the role of extracted P fraction in the P cycle in soils from different environments (climatic and weather) and land-uses.

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